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PROCEEDINGS

ELECTRON MICROSCOPY SYMPOSIUM

WEDNESDAY, FEBRUARY 3, 1982

THE BRISTOL PLACE HOTEL

TORONTO, ONTARIO

Ministry of the Environment
Ontario

The Honourable
Keith C. Norton, Q.C.,
Minister

Gérard J. M. Raymond
Deputy Minister

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Electron Microscopy Unit
Water Quality Section
Laboratory Services Branch
Ministry of the Environment
Province of Ontario

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EDITOR:

DARKA O. MIGUS

FOREWORD

The year 1939 saw the advent of the first commercial electron microscope. The field of electron microscopy has since undergone amazing growth and changes marked by a series of innovations and improvements. Advanced techniques for image optimization, automated image analysis and numerous detection systems have made the electron microscope an indispensable research tool.

Fully automated quantitative and qualitative analysis of all elements down to beryllium can be obtained using combined energy and wavelength dispersive spectroscopy. On-line image quantitation (size, shape, count and statistical data) can be derived and combined with X-ray analysis to rapidly provide invaluable information about a sample.

With such major advances occurring, this symposium was conceived to inform people of the numerous applications of electron microscopy as well as to create an awareness amongst potential users, of the capabilities and potentials of this instrumentation.

ACKNOWLEDGEMENTS

The Electron Microscopy Unit of the Water Quality Section would like to extend its appreciation to all the speakers who have contributed their time and effort in making this program possible.

Financial support from the Water Quality Section is gratefully acknowledged. Special thanks are extended to Serge Villard, Manager of the Water Quality Section, for providing support, assistance and encouragement during the planning of this symposium.

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LIST OF ABBREVIATIONS

AEM	Analytical Electron Microscopy
CRT	Cathode Ray Tube
CTEM	Conventional Transmission Electron Microscope
EDX	Energy Dispersive X-ray Analysis
EDS	Energy Dispersive Spectroscopy
EELS	Electron Energy Loss Spectroscopy
EPMA	Electron Probe Microanalyzer Electron Microprobe
SAED	Selected Area Electron Diffraction
SNR	Signal-to-Noise Ratio
SAM	Scanning Auger Electron Microprobe
SEM	Scanning Electron Microscope
STEM	Scanning Transmission Electron Microscope
TEM	Transmission Electron Microscope
WDS	Wavelength Dispersive Spectrometer

INTRODUCTION

The utilization of electron microscopy for the analysis of a wide spectrum of samples is broadening our understanding of environmental processes. The capability of "seeing" an object smaller than a dust mote and "knowing" its full chemical composition is providing insight into many diverse processes. The formation of aerosols, changes in composition and structure of air and water particulates, the growth of tubercles in water conduits and the identification of sources, transport and deposition of materials in the atmosphere, soil and water are a few of the studies which have enhanced our understanding of the environment. The utilization of this type of instrumentation is increasing yearly making it necessary to meld together the environmentalists and the user of this type of instrumentation. The exchange of information between individuals at a symposium of this nature will further both environmental science and the field of electron microscopy.

Serge Villard, Manager
Water Quality Section

THE RUDIMENTARY PRINCIPLES OF MICROSCOPY WITH ELECTRONS

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In the available time it will only be possible to skim across the surface of electron microscopy, briefly describing the functioning, purpose and types of information obtained from commercially available Electron Microscopes (EM). Indeed, the present objective is to construct a skeleton upon which later speakers will build.

Our subject consists of two broad but interdependent categories: the physical characteristics of the EM, and the high energy electron/specimen interaction. Most EM's can be placed into one of two categories:

(a) Transmission - such as the TEM (or CTEM, Conventional Transmission Electron Microscope) where the viewed area is flooded with electrons which pass through the sample (transmitted) and are subsequentially used to produce a projected image of the specimen's interior.
(b) Scanning - a fine pencil of illumination is rastered across the specimen surface such as in the SEM (Scanning Electron Microscope). Surface images (as opposed to the bulk images in transmission) are obtained.

Each technique has distinct and unique advantages depending on the information required. Recently, hybrid instruments have been produced in which both techniques have been combined to produce the STEM (Scanning Transmission Electron Microscope) and the AEM (Analytical Electron Microscope).

CTEM

The CTEM can be thought of as a linear arrangement (from top to bottom) of: An electron gun for illumination; A system of lenses and electromagnetic coils to focus and position the electron beam onto the sample; A specimen sufficiently thin to allow passage of the electrons An objective lens to form an image; A series of lens to manipulate the image; A fluorescent screen to make visible the image; and finally, A photographic recording device.

The objective lens creates both an image and a diffraction pattern (uniquely determined by the specimen orientation) either of which can be used as the 'object plane' of the following lens system. The CTEM is therefore a useful instrument for examining bulk effects, such as orientational relationships between matrices and precipitates, distribution of particles, fundamental factors affecting strength of materials, identification of phases and viruses, etc. The TEM lacks the ease to 'look' at rough surfaces for which the SEM excels.

SEM

The electron beam is focussed into a probe < 5 nm in diameter which is scanned in a rectangular raster across the specimen surface. The incident primary electrons liberate low energy secondaries, the number of which is related to the local angle between the specimen and electron beam. The secondaries in turn are collected, amplified and used to form a signal for a corresponding point on a CRT which is synchronously scanned with the sample. In this way, a high resolution image of the surface is integrated point by point (as in a TV). Magnification is controlled by changing the raster width on the sample while

that of the CRT is fixed. Focus corresponds to the minimum diameter and 'roundness' (cross-section) of the electron beam.

The SEM is used for little else other than examining rough surfaces at resolution approaching 3 to 5 nm, or for X-ray analysis (X-rays characteristic of the sample are produced by the primary electron bombardment). Diffraction patterns can be obtained from back-scattered primary electrons, however, the technique has been used sparingly because of the nature of the surfaces examined, the special instrumental conditions required and the difficulty of interpretation.

STEM

Two directions can be taken to improve image resolution in transmission, reduce CTEM instrumental aberrations or reduce STEM probe sizes to atomic dimensions. The latter also makes possible analysis of localized phenomena by stopping the beam at a particular point. Since the sequential transmitted signal can be easily manipulated electronically, many STEM's have found extensive use in fine scale X-ray and electron energy loss analyses (the electrons transmitted through the bulk suffer characteristic energy losses to produce X-rays). The STEM has limited application in the study of crystalline materials because of problems with the signal/noise ratio. The required wide collection-angle reduces - or even eliminates - many image contrast effects which microscopists rely upon for analysis.

AEM

The Analytical Electron Microscope is a response to the S/N ratio problems of the STEM since it possesses the ability to function in a CTEM or SEM/STEM mode. Of course, as the cliche goes - you can't have your cake and eat it too. In other words, though the AEM offers the advantages of both instruments, there are trade-offs which reduce instrumental performance.

The most used AEM functions are localized ($\leq$ 5 nm diameter) chemical analysis by X-rays or electron energy loss (particularly for low atomic number materials), and convergent-beam diffraction. This last technique results from the high electron beam convergence (to form a probe) and has become a powerful tool for the determination of crystal structure - say for instance, in biological macro-molecules which do not form a long-range lattice.

SAM

The Scanning Auger Electron Microscope (SAM) is similar to the SEM but analyzes the secondary electron energy. The electrons are termed Auger and are particularly abundant for light elements. Their origin lies in the absorption of the characteristic X-rays (generated by the primary beam) which stimulate the emission of less energetically bound electrons, also characteristic of the emitting atom, and which can be used for analysis. Because of their low energy, only those emitted from very close to the surface can be detected (remainder absorbed). Surfaces to be analyzed must be clean (otherwise the contamination is analyzed). Because of the inherently low signal, the technique is difficult.

Future

In the future, the AEM will probably be further developed and extended to higher electron accelerating voltages, which theory promises will yield more accurate and better results. Computer software for the analysis of electron energy loss spectrometry will be improved as well as the development of parallel detectors which will allow the simultaneous recording of more than one X-ray or energy-loss level.

INTRODUCTION TO ELECTRON BEAM INSTRUMENTS

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Today's technology requires that the scientist observe and correctly explain phenomenon occurring on a micron (1um = 10^{-6}m) and submicron scale. Since this task is beyond the physical limits of the light microscope a new science involving higher energy particles, namely electrons, was developed. Electron beam instruments now permit characterization of heterogeneous materials and surfaces on the level of tens of angstroms (1Å = 10^{-9}m). In this paper we will discuss the basics of the electron beam forming optics and the methods by which a vast amount of information is obtained from the electron beam - specimen interaction.

A. ELECTRON OPTICS - In order to resolve features on the order of 10Å the electron beam must strike the sample in a very finely focussed spot. The technology developed to produce such a beam will be reviewed here, but for a more defined treatment see Zworykin et al[1]. Firstly we must note that since electrons interact strongly with air molecules it will be necessary to produce the electron beam enclosed within a column at very low pressure (on the order of 10^{-6} Torr). The source electrons are normally produced by passing a current through a tungsten filament such that at very high temperatures some electrons become energetic enough to overcome the work function and escape the source. This process is termed thermionic emission and the assembly in which it occurs is the electron gun consisting of a tungsten filament and a Wehnelt cylinder (see figure 1). An electron beam is then formed by accelerating this cloud of electrons toward a high potential target called an anode plate. The Wehnelt cylinder is then biased at a negative potential relative to the filament and the beam is focused to a crossover point somewhere between the gun and the anode. At this point an image of the filament tip will be formed on the order of 30 um in diameter. It is this image which will undergo a series of demagnifications and eventually be projected onto the sample surface. Thus, it will be necessary to achieve demagnifications of around 10,000 to achieve a final spot size of 30Å. As in an optical microscope a combination of condenser and objective lenses must be used to achieve the final demagnification. The lenses in an electron beam instrument utilize the physical law that the path of a moving electron is charged as it passes through a magnetic field. The force F experienced by an electron moving with a velocity V in a magnetic field H is given by the equation

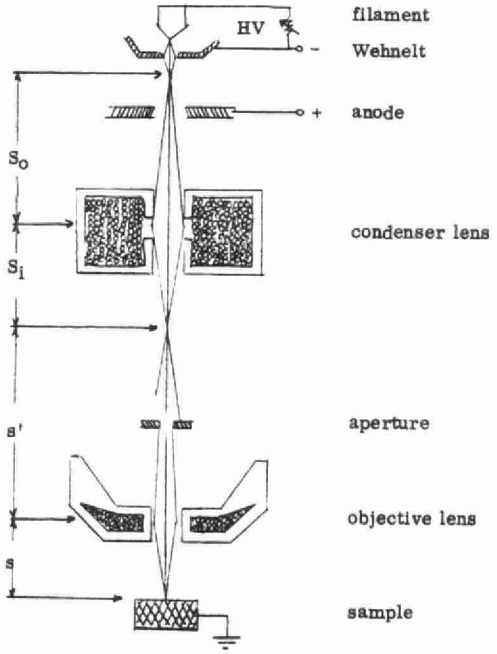

FIGURE 1. Schmatic of typical electron microscope optical column.

$$F = -e(V \times H)$$

where e is the charge on the electron. The electromagnetic lens is produced by an electromagnet consisting of a steel housing and a core of wire windings. It is rotationally symmetric and wound so that electrons are focused toward the central axis of the column. The intensity of the magnetic field produced by the lens is such that electrons travelling down the exact center of the lens are unaffected

while electrons displaced from the center are focused toward the central axis, much in the same manner as light is focused by a double convex lens. The strength of an electromagnetic lens is proportional to NI, the number of windings times the current passed through the lens. Since the focal length is proportional to the strength of the lens it can be changed by varying the current passed through the windings[2]. The ray diagram shown in figure 1 illustrates the path of the electrons as they pass from the crossover point through the condenser and objective lenses to the sample. The thin lens equation from light optics applies here, yielding the relationship

$$(1/S_o) + (1/S_i) = 1/f'$$

where f' is the focal length of the lens. The demagnification of such a lens is given by the equation $M = S_o/S_i$, and for the ray diagram in figure 1 is greater than one. The total demagnification of the two lens system is the product of the demagnification of each lens, and the final spot size is equal to d_o divided by the total demagnification. Using the two lens column as illustrated in figure 1 one can vary the beam current by adjusting the condenser lens strength and focus the spot size by adjusting the objective lens[3]. Just as in the case of light optics, electromagnetic lenses suffer from inherent aberrations which limit the minimum spot size. Basically three types of aberrations are present in the electron optical system, namely, spherical aberration, chromatic aberrations and astigmatism. Spherical aberration arises because electrons moving in paths further away from the central axis are focused more strongly than those near the axis. This has the effect of blurring the focal point of the lens into a disc of confusion in the image plane. Chromatic aberration is a result of the statistical spread in energy of the electrons in the beam and hence a spread of electron velocities. This spread of velocities again produces a larger disc of confusion at the image plane. Astigmatism is seen as an elliptical spot rather than a round one and is due mainly to the fact that the electromagnetic lenses lack true radical symmetry. Clearly there is very little one can do about chromatic and spherical aberration however astigmatism can be corrected by using a series of electromagnetic coils which can be excited to shape the beam into a perfect circle.

One of the most important advantages of the electron beam instrument is the large depth of field which is attainable. This characteristic is a direct result of the very small divergence angle made by the electron beam as it travels through the lenses. At comparable magnifications, the depth of field of an electron beam instrument is over 100 times greater than that of the light microscope. Generally a set of scan coils are used to raster the electron beam over the sample and display the signal detected on a synchronous scan CRT. This allows the spacial distribution of information to be visualized on a 2-dimensional screen. The subsequent magnification can then be changed by changing the size of the area rastered on the sample.

B. ELECTRON BEAM-SPECIMEN INTERACTION - It is the result of the interaction of the electron beam with the sample which yields information characteristic of the material. This information is signaled from the sample to a detector in the form of one of several types of particles. Among the signals produced are secondary electrons, backscattered electrons, characteristic x-rays, Auger electrons and photons (light) of differing energies. Each of these particles carries with it unique bits of information concerning the material directly underneath the sample-electron beam intersection point. A simplistic view of this interaction is that the electron beam strikes the sample and penetrates to a certain depth below the surface producing the signals mentioned above. These signals escape from the sample if they are energetic enough to make it to the surface without being absorbed. Figure 2 depicts a cross section of the volume excited by the electron beam and shows the general depth from which each signal comes. Auger electrons generally have energies of a few hundred electron volts and thus only escape from the first couple of atomic layers whereas characteristic x-rays can have energies of up to 20,000 electron volts and can escape from as deep as 10 microns. The range, or depth, from which the particle originates not only determines the information carried but the spacial resolution of each signal. A brief description

of the origin and the type of information available from each signal follows; (for a more detailed treatment see Goldstein)

1. Secondary Electrons - Secondary electrons are low - energy electrons (50eV) which are formed as a result of excitation by the high energy primary beam of loosely bound atomic electrons. Due to the low energy of secondary electrons the depth from which they come is very shallow and hence the resolution is very close to the diameter of the focused electron beam. The major influences on the number of secondary electrons produced are the topography of the sample surface, the local electric potentials which are present on or above the sample surfaces and variations in mean atomic number across the sample. Secondaries are collected on a biased phosphor screen connected to a photomultiplier tube. The signal then modulates the brightness of a CRT scanned in phase with the beam (see figure 3a).

2. Backscattered Electrons - Backscattered electrons are produced by large-angle single elastic scattering events and by small-angle multiple elastic scattering events. The mean energy of backscattered electrons vary between 50% to 98% of the beam energy. The backscattered yield is dependent upon the mean atomic number of the sample as well as the angle from at which the electron was ejected from the sample. Therefore the information received from the backscattered signal is mainly concerned with the variation in atomic number across the sample. Special solid state detectors are available which separate compositional data from topographic and display either on a CRT. These electrons come from a greater depth than secondaries and hence do not display good spacial resolution (see figures 3b and 3c).

3. Auger Electrons - Auger electrons are a result of a relaxation of outter shell electrons into inner shell vacancies. Such a deexcitation is accomplished by a loss of energy in the form of an x-ray or Auger electron. The energy of the Auger electron is characteristic of the element from which it came. Therefore, by measuring the number and energy of the Auger electrons, one can identify the elements present in the sample as well as the amount of the particular element present. Since Auger electrons are very low energy particles the information is representative of the first few atomic layers.

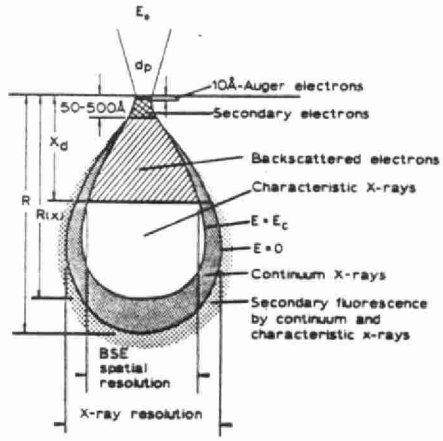

FIGURE 2. Summary of range and spacial resolution of backscattered electrons, secondary electrons, x-rays and Auger electrons.

4. Characteristic X-rays - X-rays, as in the case of Augers, come from the transition of electrons from outer shells to inner shells. X-rays have energies and wavelengths characteristic of the elements from which they came. X-rays are used to determine the chemical composition of the sample directly beneath the beam[5]. The x-ray signal can be collected on either energy dispersive detectors (EDS) or wavelength dispersive spectrometers (WDS) and then displayed on the CRT in the form of a "dot map" (see figure 3e).

5. Cathodoluminescence - In certain types of materials, the interaction of high energy electrons creates electron-hole pairs. If the electron-hole pairs recombine, energy is given off in the form of long-wavelength radiation commonly referred to as cathodoluminescence. Quantitative data can be obtained by recording the emitted spectral intensity as a function of wavelength or photon energy. The signal can be obtained from a photomultiplier and used to modulate the brightness of the CRT so as to obtain an image of the emitting surface[4]. Again, since the light comes from a depth of a few microns the resolution is very poor (see figure 3d).

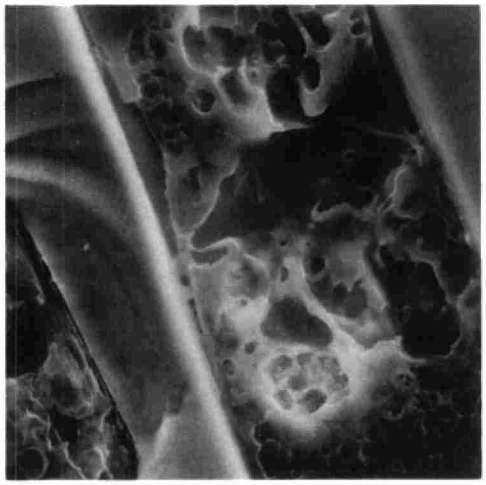

Figure 3a. Secondary electron image.

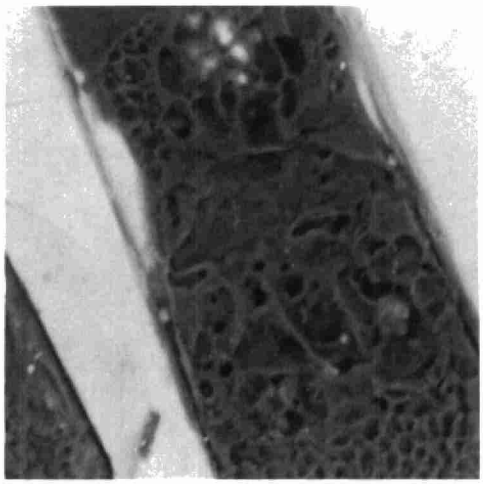

Figure 3b. Backscattered electron image. (compositional)

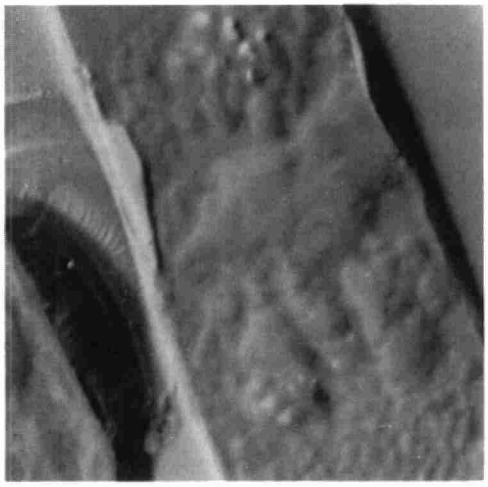

Figure 3c. Backscattered electron image. (topography)

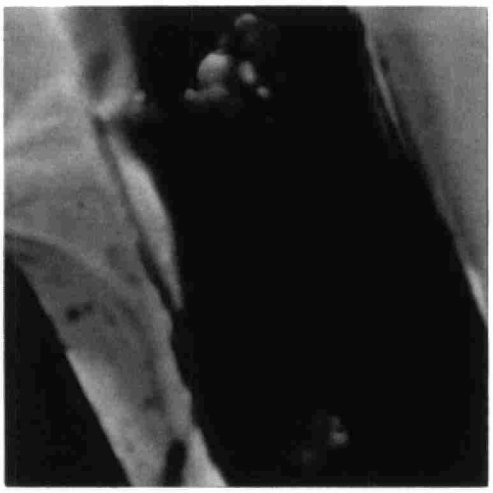

Figure 3d. Cathodoluminescence image.

Figure 3e. X-ray dot map image.

Figure 3f. Absorbed electron image.

6. Absorbed Current - As the electron beam strikes the sample a fraction of the incident electrons are backscattered the rest are absorbed. The fraction which are absorbed depends on the average atomic number of the material; the lower the atomic number the higher the fraction absorbed. The absorbed current signal is obtained by amplifing the specimen current and modulating the CRT brightness (see figure 3f).

C. SUMMARY - It is a very ambitious task to attempt to cover all aspects of electron microscopy in such limited space. In fact it's impossible; we have therefore touched just the high points and omitted several important functions. Among those topics left out the most encompassing is transmission electron microscopy. TEM deals with the information obtained by electrons as they penetrate completely through the sample and is the subject of thousands of reasearch publications. What we have attempted to do here is introduce the reader to the fundamentals of scanning electron microscopy (SEM) stressing theory of operation and the types of information available. It should be clear that the SEM is a very powerful tool which is finding new applications in almost every line of work. From the R & D labs of every major industrial company to the production lines of small manufacturers SEM's are proving to be extremely useful and invaluable instruments.

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ANALYTICAL INFORMATION FROM ELECTRON MICROSCOPY

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When the electron beam of an electron microscope strikes a specimen a number of interactions may occur. Several of these interactions can be used to provide useful information about the specimen. The following sections consider a number of the ways in which electron microscopes can provide information about the physical nature of the specimen.

A. Electron Diffraction:

Since the wave nature of the electron beam is similar to that of light, electron waves hitting a regularly structured specimen, such as a crystal, can be observed on the screen of a TEM and recorded on the photographic film in the microscope. The electron diffraction pattern appears as a series of spots when the electron beam is diffracted through a single crystal and as a set of concentric rings radiating out from the centre when the beam is diffracted through several crystals. One can identify an unknown compound by comparing the electron diffraction patterns of known chemical compounds to the unknown.

Electron diffraction has some advantages over X-ray diffraction because it requires less material, because it can detect deposits as small as 2 μm or less in diameter and because the crystalline deposit is still spatially located in the sample. Electron diffraction has disadvantages including problems in thin sectioning of crystalline deposits and damage due to heating. For example calcium oxalate converts to calcium oxide due to heat produced by the electron beam. Electron diffraction is used extensively in metallurgy but its use in studying biological tissues is limited. For more information consult:

> Electron Diffraction and Optical Diffraction Techniques by B.E.P. BEESTON, R.W. HORNE and R. MARKHAM. 1973.

B. X-ray Analysis:

An atom has a positively charged nucleus around which are shells of negatively charged electrons. These shells, which are labelled K, L, M, N, O, etc., may hold a fixed number of electrons. For X-ray fluorescence to occur an electron must first be ejected from an inner shell. In order for the atom to revert to energy stability, the vacancy is replaced by an electron from an outer shell transferring to the now vacant site. A number of such inter-orbital transitions are possible. When an outer shell electron falls to an inner shell position the energy difference is released, perhaps in the form of an X-ray. This X-ray will have a definite energy, dependent upon the type of atom involved and the inter-orbital transition that occurred. The energy aspect of the radiation released forms the basis of X-ray energy spectroscopy. The radiation released has a dual property however, since it also has a wave property. This wave property forms the basis for wavelength dispersive spectroscopy of X-rays.

For X-ray analysis in an electron microscope, a selected area of a specimen is bombarded by an electron beam of sufficient energy to bring about ionization of atoms in that area of the sample. The intensity of X-rays produced by different elements is dependent upon several factors including the atomic numbers of the elements involved, the accelerating voltage, and the beam current used in the microscope.

The most intense X-ray lines are the K lines which are produced following ejection

of K shell electrons. Similarly L lines and M lines are produced following ejection of electrons from L shells and M shells respectively. Within shells there are several possible electron jumps. Thus within the K lines, for example, there are in descending order of intensity, $K_{\alpha 1}$, $K_{\alpha 2}$, $K_{\beta 1}$, $K_{\beta 2}$, lines.

For X-ray analysis studies of biological tissue a major problem is specimen preparation. Where tissue must be treated with fixatives and dehydrating solutions there is always concern that material may have been extracted or moved from one area to another. For more details on specimen preparation methods consult the book by Chandler, 1977.

1. Wavelength Dispersive Spectroscopy:

Wavelength dispersive spectrometers measure the wavelengths of X-rays produced when an electron beam hits a sample. X-rays are reflected by a curved crystal of known lattice spacing. Since only one particular wavelength is strongly reflected at a certain angle it is possible to calculate the wavelength of the X-rays. With this system only one wavelength can be measured at a time.

2. Energy Dispersive X-ray Analysis:

Energy dispersive X-ray analysis measures the energy levels of X-rays entering the detector. X-rays pass through a beryllium window into a solid state detector immersed in liquid nitrogen. The charge produced in the detector by each X-ray is collected, passed on to a preamplifier, further amplified, and passed on to a multichannel analyzer. In the multichannel analyzer the pulses are separated in terms of amplitude and stored in memory channels that correspond to these amplitudes. The resulting spectrum is often displayed on a cathode ray tube but may also be stored in various ways. A number of computer manipulations of the spectrum are possible.

For interpretation of EDX analysis spectra several features are important. Across the bottom of the spectra will be numbers that are the energy levels in kiloelectron volts. The energy ranges generally will be chosen to allow maximum separation of peaks of the elements present. From standards the position of elements can be determined. For example, the major peak for Ca is the $K_{\alpha 1,2}$ peak at 3.690 keV. The calcium K_{β} peak, which is 10% of the K_{α} peak, is located at 4.102 keV. The spectrum will also show an analysis time in seconds and a vertical scale. The larger the vertical scale number the bigger the scale.

Energy dispersive X-ray analysis has several advantages over wavelength dispersive X-ray analysis for biological studies. Some advantages of EDX analysis systems include: having a much higher efficiency, being able to analyze all elements Na and heavier simultaneously, being cheaper to buy and easier to maintain, the ease of identification of peaks and the ease of computer processing of the peaks (smoothing, background subtraction, calculation of integrated peak heights, etc.). Since biological samples often are damaged to some degree by the intense electron beam, having simultaneous analysis of all elements is very useful. For certain quantitative studies, especially involving trace elements, the wavelength dispersive systems are superior however.

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C. Electron Energy Loss Spectroscopy (EELS):

Electron energy loss spectroscopy, which is the study of the energy distribution of electrons which have interacted with the specimen as they passed through it, can give quantitative information about the chemical and physical nature of the sample. To obtain an EELS spectrum the electrons leaving the thin specimen are passed into a spectrometer which disperses it into various energy components. The spectrometer makes use of a "magnetic prism" to bring about the separation. Electrons that have lost considerable energy in passing through the sample will be more influenced by a magnetic field and hence will be bent to a different extent than electrons that did not loose any energy passing through the sample. If a slit is placed in the image plane, electrons of any given energy loss can be selected and a spectrum formed.

While the use of EELS in biology is in its infancy, this method is one that certainly will become more important in the future. EELS is a particularly useful method since it is very sensitive and can study the light elements that EDX analysis cannot. Certain parts of the EELS spectra may allow a "finger printing" of complex biological molecules and localization with a resolution better than $100\,\mathring{A}$. In some cases (such as fluorine) it may be possible to detect single atoms. Development of stains that are identifiable by their energy loss characteristics is a certainty. Numerous sample preparation problems are yet to be investigated but certainly the need for a very thin (33-50NM) section is a limitation for some studies as is radiation damage.

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IDENTIFICATION BY TRANSMISSION ELECTRON MICROSCOPY OF FRESHWATER ALGAE

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Algae are the smallest and most primitive of the plants on earth and are the foundation or the "building blocks" of aquatic food webs. Without algae there would be no fish and many other vertebrates including several birds and mammals which are dependent on fish for food. Taxonomy is the science of naming and identifying species. This has been done in an organized way so that organisms with similar structural and/or life history characteristics are grouped together. The result is a hierarchial system of organization such that groupings or associations of species are formed which tend to follow similar evolutionary lines and possess similar ecological requirements and tolerances. Taxonomy therefore, is the foundation of ecology. Taxonomy is a requisite of any investigation of algae related to aquatic food web function, influence on domestic drinking water supplies or recreational water quality.

Much of the work of the original algal taxonomists such as Ehrenberg, Dujardin, Perty, Keutzsing, Lemmerman and others, who were active between 1830 and 1910, is still valid today. Presently, there exist some 13,800 species of freshwater algae. The taxonomy of more than 10,000 of these is solidly based on light microscopy (LM) of cell structures and life history. Soon after the invention and refinement of the electron microscope during the 1930's, the first observations of algal cells by electron microscopy (EM) were reported. The use of EM in algal taxonomy has been most conspicuous for the single class Chrysophyceae since members of this group have several features which 1) are now recognized as very important identifying characters and 2) can be recognized easily only with EM. Another reason for stressing the Chrysophyceae in this presentation is their widespread and important distribution in Ontario lakes where they often comprise more than 50% of the average annual phytoplankton biomass.

Most members of the Chrysophyceae have flagella during some stage of their life history. The relative lengths and structure of the flagella as seen with the electron microscope, as well as two or three additional cell characteristics serves to organize the class into about 150 genera and 800 species. Brown (1945) and Petersen (1949) were the first to demonstrate with EM that the longer flagellum of chrysomonads was adorned with fine filaments called mastigonemes. EM of sectioned flagella (Manton & Leedale 1961) showed the shaft was constructed of 9 paired periferal tubules and 2 central tubules. Other flagellate species, which had previously been assigned to the Chrysophyceae for reasons based on LM, were investigated by EM during the 1955-1962 years and it was shown that one of the "flagella" of these organisms was not really a flagellum but an organ of very different structure and function (now known as a haptonema). Species of this group, which all possess a covering of organic and/or calcified scales, have since been separated taxonomically from the Chrysophyceae and include several taxa of economic importance. For example, Prymnesium parvum which is a toxin producer is known to regularly cause massive fish-kills in Europe, Britain and the Middle East. In North America, Prymnesium's close relative Chrysochromulina breviturrita has caused serious odour production in several lakes in New England and Ontario. These species can be identified with certainty only with EM owing to the distinctive pattern on the tiny (<1 µm diam.) organic scales which cover the cells.

Scale structure is the most important identifying feature in the family Synuraceae which includes 10 genera and about 130 species, none of which can be positively identified by LM because the siliceous scales which cover the cells are ornamented with patterns which can only be resolved by EM. The widespread use of EM for the identification of species in this group has expanded considerably since the first electron micrographs were published in Czechoslovakia and Denmark in 1955. The largest genus is Mallomonas with about 70 species now confirmed by EM; about 30 of these have been described during the past 20 years.

In Ontario, about 40 species of Mallomonas have been identified by EM. Many of these have not previously been reported from North America and several others are apparently undescribed and unnamed species. Several examples of scales from species in Ontario and other parts of the world illustrate the reliability of scale morphology "seen" with the electron microscope as a taxonomic criterion. Studies of this group are continuing in Ontario because of the apparently useful "acid-indicator" quality of many species. It is apparent from work done so far that several species are found only in acidic habitats; others are restricted to hardwater lakes while others appear to be indifferent to acid status since they are found in a variety of lake types including a wide range of lake pH. Owing to their highly refractive siliceous structure, the scales preserve well in lake sediments and historical change in lakewater pH may be inferred from changes in scale (species) composition in lake sediment cores. In the absence of long term chemical data, the chrysophycean scales of lake sediments should indicate the rate of acidification of selected lakes during the recent past.

Unfortunately, the use of the electron microscope has also led to some confusion in that some species recently described by EM cannot be cross-referenced to older descriptions based on LM. As a result, two species descriptions and two different species names have in some instances been assigned to a single organism. In most cases there exist no archived collections of the original material described by LM, so these difficulties will probably never be satisfactorily resolved. These problems are minor in contrast to the tremendous advances in taxonomy which the electron microscope has given to the aquatic scientist confronted with the identification of microscopic algae.

A BUD DEVELOPMENT STUDY ON ONE-YEAR-OLD CONTAINER-GROWN CONIFER SEEDLINGS

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Containerized tree seedlings are now a significant part of the planting stock production system, which plays an important role in Ontario's regeneration program. In 1981 approximately 14 million containerized seedlings were produced and this is expected to be increased to 40 million by 1985, while none were produced in 1960.

A major advantage of the container-grown seedling over the conventional bare-rooted seedling is that the roots of the former are undisturbed and protected at the time of outplanting. At one time it was thought that the container seedling would replace the bare-rooted seedling but each stock-type has certain advantages. Both are accepted stock types that are successfully used in many areas of Ontario.

Containerized tree seedlings are generally grown in greenhouses and frequently two crops per year are produced, between March and August. This growing environment is, therefore, relatively artificial, but can be controlled to some degree to influence the growth pattern of the seedlings. Proper bud development is necessary for the seedlings to survive the winter and to enhance subsequent growth after outplanting. In particular, what are the effects on growth cessation and bud development towards the end of the growing season.

A study was, therefore, initiated in August 1980 to determine just how buds developed and to find ways of improving bud development, if necessary. This study is currently being conducted by Mr. S.J. Colombo (1) at the Swastika (Lat. N 48°06', Long. 80°06') and Thessalon (Lat. N 46°15', Long. 83°34') tree nurseries, which are two of our Ministry's major production centres for container-grown stock. Although the study deals with 3 species, black spruce (Picea mariana (Mill.) B.S.P.), white spruce (P. glauca (Moench) Voss) and jack pine (Pinus banksiana Lamb.), only the black spruce results will be discussed here.

Monitoring of bud development started on August 14, 1980 on regular, production-run container-grown black spruce seedlings, sown around the end of May 1980, and placed in their overwintering beds at Swastika nursery at the beginning of August. About 25 seedlings were collected at approximately weekly intervals until the end of October.

Bud development was determined by dissecting the shoot apices. First the bud scales were removed to reveal the apical meristem upon which needle primordia had formed. Then, with the use of a dissecting microscope, small numbers of needle primordia (±30) were directly counted, whereas larger numbers were calculated by counting the number in two or three spiral parastichies (rows) arranged around the apical meristem and multiplied by the number of rows.

Bud initiation had already started by August 14, when day length was about 14½ hours and an average of 15 needle primordia had already formed. Primordia formation had stopped by September 25 and an average of 95 primordia had formed leaving large portions of the apical dome bare (Fig. 1).

During the following winter, Mr. Colombo grew spruce seedlings from seed under environmentally controlled optimum conditions for 12 weeks. Subsequently, these seedlings were placed under a 8-hour photoperiod and at a temperature of 20°C, which are optimum conditions for bud development (1). Under these conditions bud development was completed in 8 weeks and well over 200 needle primordia had formed, which covered nearly all of the apical meristem (Fig. 2).

There is, therefore, room for improvement in bud development of production-run container stock. Consequently, in late July 1981, some production-run stock was placed under an 8-hour photoperiod and some was given extended greenhouse culture to maintain higher temperatures, i.e. closer to the optimum during the slower but naturally decreasing photoperiod. The preliminary results show that both treatments give improved bud development.

Graphs have been plotted (Fig. 3) to show rates of primordia formation but none has the impact of a scanning electron-photomicrograph, such as the one in Fig. 1 or 2. Incidently, the photographs were taken by Dr. P. Roberts of this section of the Ontario Ministry of the Environment. Seeing is believing! It is the age-old adage

that one picture, in this case an electron-photomicrograph one, is worth a thousand words or a thousand graphs for that matter.

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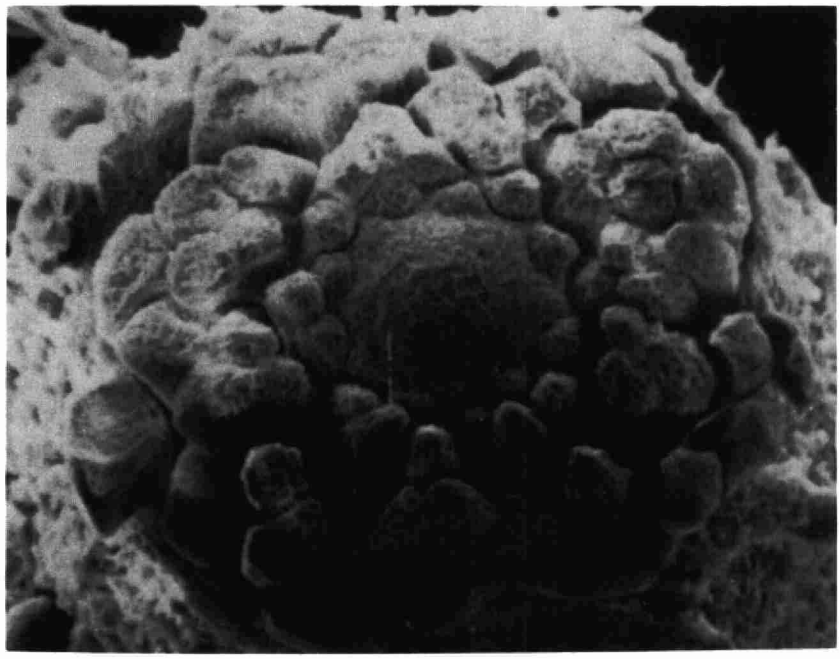

Figure 1. Scanning electron-photomicrograph of a representative bud from a production-run spruce container seedling. (95X)

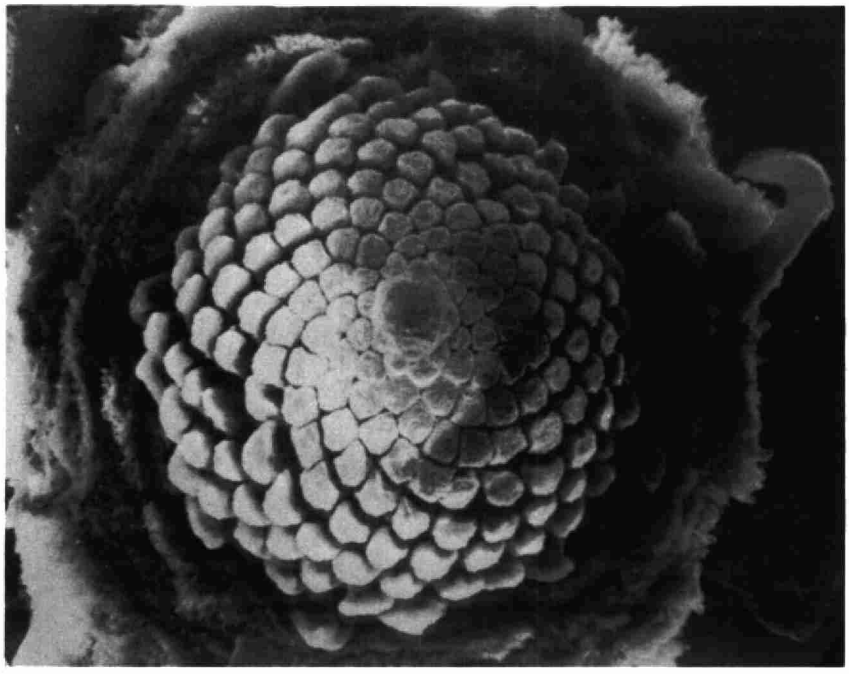

Figure 2. Scanning electron-photomicrograph of a representative bud from a spruce seedling that was formed under optimum conditions. (60X)

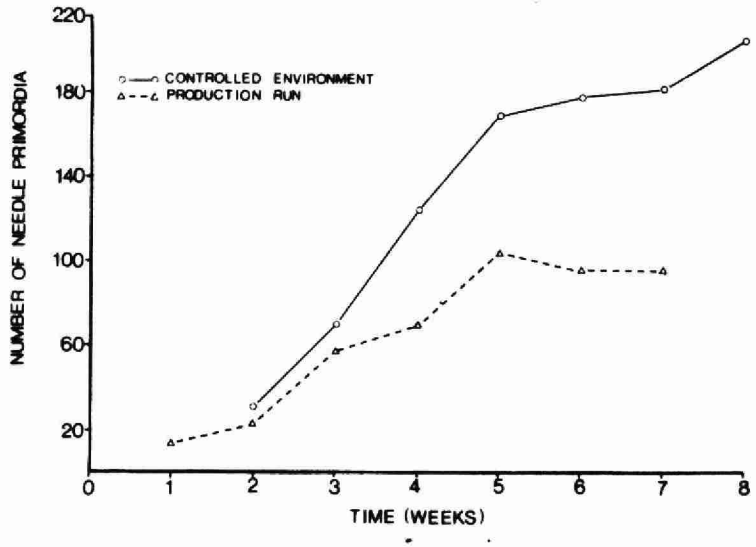

Figure 3. Needle primordia initiation in black spruce under controlled conditions (8h day; 20°C) and under production run (natural) conditions.

ELECTRON MICROSCOPY IN THE STUDY OF VIRUSES AND VIRUS DISEASES

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The extremely small size of viruses (approximately 20-300 nm) makes the electron microscope an essential tool in the study of viral ultrastructure. Relying primarily on the simple negative staining technique in combination with transmission electron microscopy, a great deal of knowledge has been accumulated on the morphology of individual viruses. It has been found that the majority of viruses consist of a core of nucleic acid surrounded by a shell or capsid of protein. The capsid symmetry is a characteristic feature of each virus family, and may be icosahedral or helical. Many well-known viruses, such as poliovirus, herpes simplex virus and wart virus, exhibit icosahedral symmetry, and their construction resembles that of Buckminster Fuller's geodesic domes.

Viruses cause infection by invading susceptible cells and converting them into factories for the production of viral progeny. Both the transmission electron microscope and the scanning electron microscope can be used to study virus-cell interactions. The sequence of intracellular events associated with viral infection in the body can be studied in tissue culture, as the changes seen in vivo and in vitro are essentially similar, and are characteristic of each virus family.

Knowledge of viral ultrastructure and virus-cell interactions can be of use to the diagnostic virologist, and increasingly electron microscopes are being employed in hospital and public health laboratories. Viruses contained in clinical specimens, or viruses isolated in tissue culture from clinical specimens, can be identified on the basis of the morphology observed by electron microscopy.

A major limitation to virus identification by direct electron microscopy is the extremely small sample size that can be examined in the instrument, necessitating a high concentration of virus in the original specimen. Much of the developmental work in this field deals with improving the sensitivity of virus detection by electron microscopy. One procedure used by our laboratory to detect viruses in environmental samples has been to expose the samples to pooled human immune serum globulin (gamma globulin) prior to examination. Antiviral antibodies present in the gamma globulin tend to concentrate any viruses present in the sample, increasing the sensitivity of detection by 50-100 times.

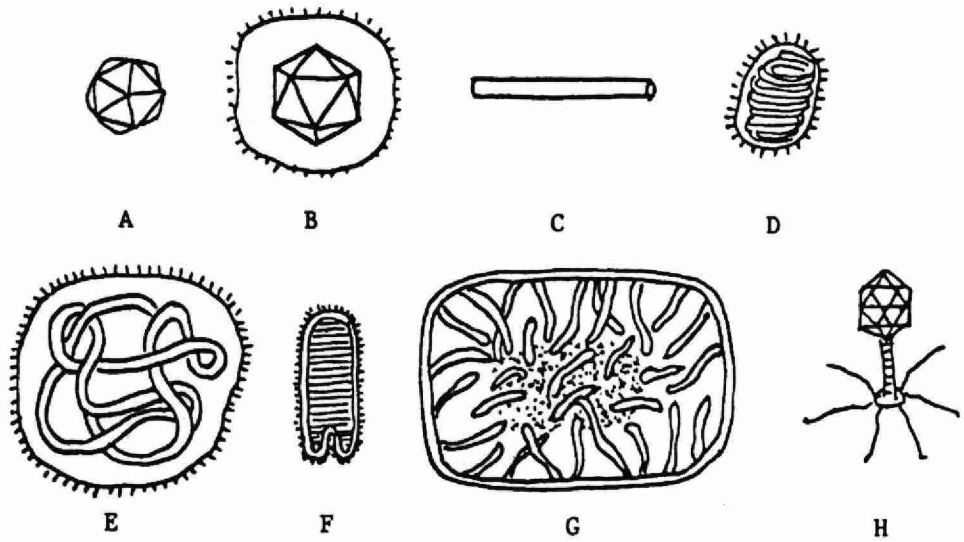

Fig. 1. Morphology of some well-known viruses. **Icosahedral symmetry**: (A) polio, wart, adeno, rota; (B) herpesviruses. **Helical symmetry**: (C) tobacco mosaic virus; (D) influenza; (E) measles, mumps, parainfluenza; (F) rabies. **Complex or uncertain symmetry**: (G) pox-viruses; (H) T-even bacteriophages.

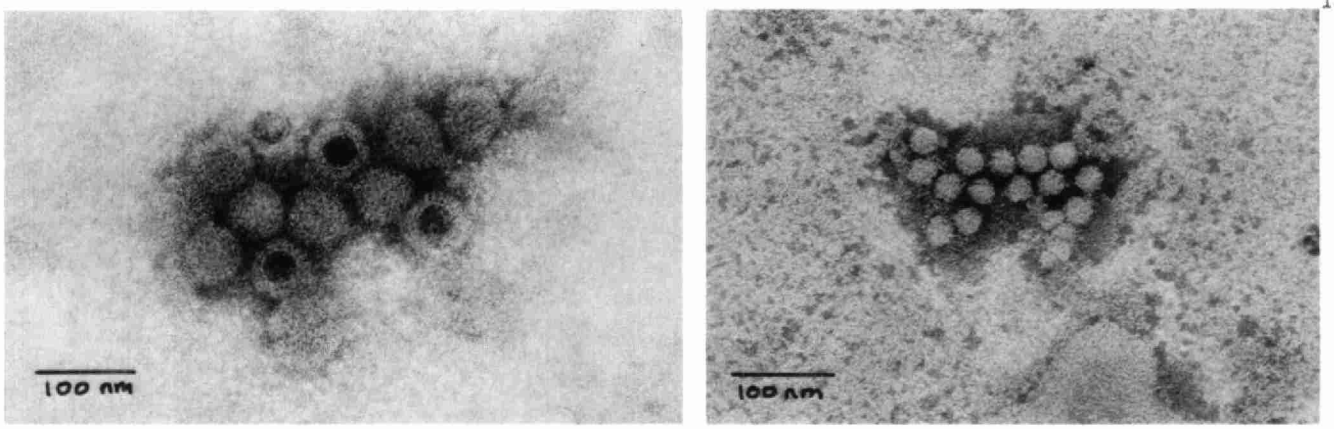

Fig. 2. Negatively stained viruses isolated in cell culture from environmental samples. *Left:* Reovirus; the negative stain has penetrated some of the virus particles, giving them an electron-dense core. *Right:* Poliovirus; these 27 nm viruses are among the smallest known viruses.

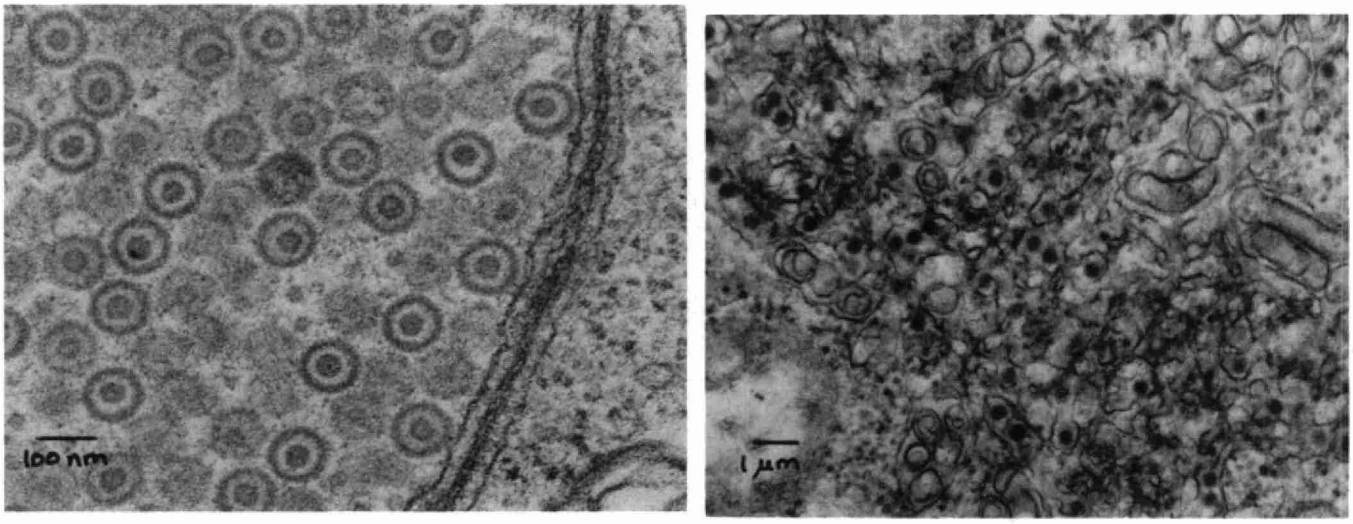

Fig. 3. Thin sections of cells infected with viruses from clinical specimens. *Left:* Herpes simplex virus; progeny nucleocapsids are seen assembling in the nucleus. *Right:* St. Louis encephalitis virus originating from a patient with suspected viral encephalitis. The dense virus particles are recognized by their size and their unique association with cytoplasmic tubules.

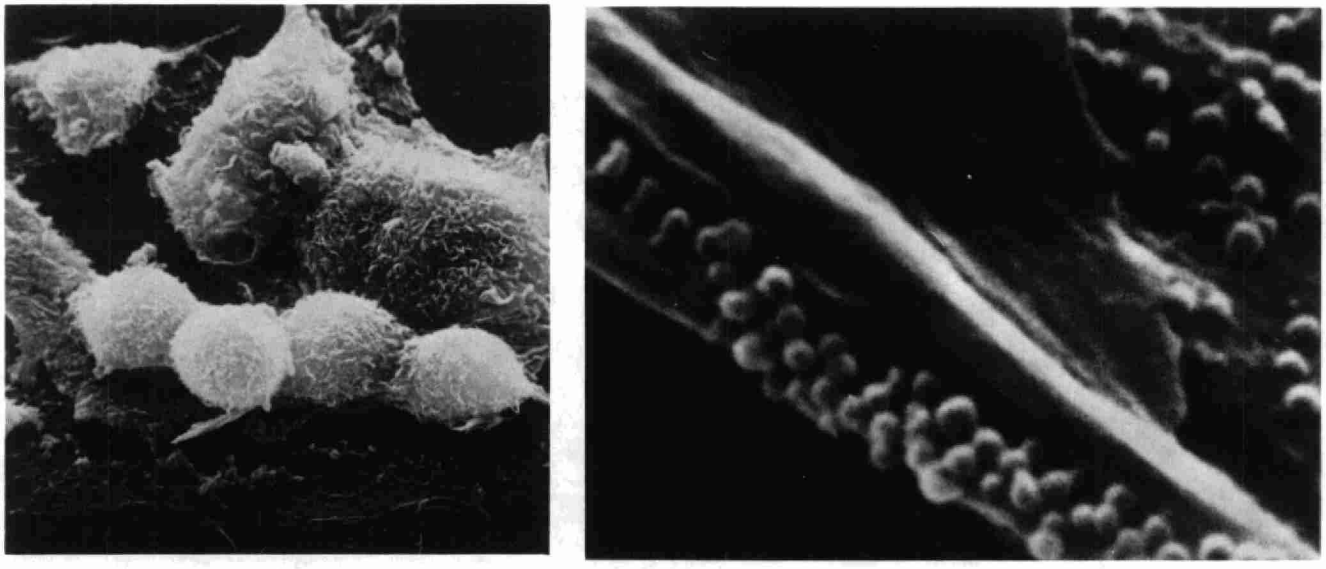

Fig. 4. Scanning electron microscopy of cell cultures infected with herpes simplex virus. *Left:*Low magnification showing rounded and distorted cells. *Right:*Higher magnification of cell surface showing viruses being released from the cell.

THE USE OF ELECTRON MICROSCOPY IN THE STUDY OF BACTERIAL WALL ULTRASTRUCTURE AND PHYSICOCHEMISTRY

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1. Metal Binding and Environmental Transport by Bacterial Polymers

As a consequence of industrial loading, wide use of synthetic fertilizers, and acid leaching of natural sources, the types and concentrations of toxic metals have increased in our environment. For example, the average content of soluble uranium in our rivers has been estimated now to be approximately 0.3 ug/litre (17). Recently there has been an increasing awareness that much of the soluble metal in our environment is actively chelated by organic members to form sedimentable, metal-rich particulates which must produce natural "sinks" of toxic metal in the environment (4, 10). The transport mechanisms of toxic metal and their various organic derivatives are therefore complicated and need to be defined.

Bacteria are ubiquitous throughout nature and consist of a variety of highly charged homo- and heteropolymers, some of which (e.g. those making up walls) are remarkably resilient to degradation (1). Cell walls have a high anionic charge density and consequently interact strongly with electropositive metal ions derived from the environment to accumulate large quantities of bound metal (1-9, 11). In fact, traces of microbes and high concentrations of associated metal have been found in sediments from ancient geological horizons (13, 15, 16). More recently, microorganisms have been implicated in the concentration of radioelements such as uranium, plutonium and polonium from marine water (14).

For several years bacterial walls have been used by us as a model system to accurately identify the steps in metal binding to biological material (1-9). These are good systems since the structure and chemistry of the various walls used in the studies are well known (4, 12) and subtle alterations to the walls are possible through the use of mutants and/or change in the growth conditions (6, 7, 9). The Bacillus subtilis 168 strain has already been accurately defined and three other system (Bacillus licheniformis NCTC 6346 his$^-$, Escherichia coli AB264, and Sporosarcina ureae ATCC 13881) are actively being studied. Each of these wall systems has been chosen because of intrinsic chemical and structural characteristics in an attempt to outline distinct fundamental principles of metal binding, each peculiar to the wall type.

In order to determine accurate sites of metal deposition within the wall fabric and to distinguish between phosphodiester, carboxylate and amine groups and their metal binding capacity, extensive use of electron microscopy, atomic absorption (A.A.), X-ray fluorescence (XRF), and X-ray diffraction (XRD) is required. Application of energy dispersive X-ray analysis and electron energy loss spectroscopy using a high resolution analytical scanning transmission electron microscope (STEM) provides quantitative information and "maps" the site of metals within individual wall fragments. These results can then be compared to the data derived from bulk analyses using A.A. and XRF.

2. High Resolution Structural Analysis of Bacterial Walls

Protein arrays which are the outermost component of some bacterial walls are being studied extensively in our laboratory. Several different paracrystalline formats are available (18) and each array is amenable to ultra-high-resolution electron microscopy. Low dose electron microscopy (ca. 300 coulombs using bright field CTEM) allows for the highest possible resolution of negatively stained wall fragments (1.1 nm resolution is possible), whereas a high resolution STEM unit equipped with a cryo-transfer and specimen holder allows 2.5 nm resolution of unstained, frozen (hydrated) wall fragments which are imaged in the STEM darkfield mode. A technique has been developed where transmission electron microscopy, electron diffraction, optical diffraction and computer Fourier analysis have been combined to produce highly clarified images of wall

structure. Using this system, two arrays have been resolved to the highest possible degree using conventional electron microscopy (3, 19, 20).

The cryo-specimen holder and transfer unit of the STEM allows a sample to be quick-frozen to maintain accurate structure, to be transfered into the STEM without ice crystal formation, and to be maintained in a frozen (hydrated) glass (all procedures at -196^{0}C) for observation, thereby maintaining a proper aqueous environment for "correct" structural observation. It is hoped that sublimation can be accurately controlled by adjustment of the specimen temperature, thereby allowing the effect of water concentration on specimen structure to be monitored. Two advantages of STEM are that the scanning mode drastically reduces electron bombardment to the specimen (i.e. structure is preserved) and that STEM darkfield detectors allow the imaging of unstained material. This should allow more accurate imaging of the protein arrays and delicate structures with smaller lattice constants (e.g. the peptidoglycan sacculus) may also be resolved. For the first time with this instrumentation, it may be possible to dissect wall structure into chemical terms by electron microscopy.

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ELECTRON MICROPROBE X-RAY ANALYSIS OF THE SEASONAL GROWTH ZONES IN FISH CALCIFIED TISSUE

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The electron microprobe X-ray analyzer was used to conduct direct analyses of the chemical composition of calcified tissue of fishes. Analytical capabilities of the microprobe provided microtopographic elemental descriptions of the qualitative and quantitative nature of the checks in the surface configurations on the scales and of optically different zones in other calcified structures.

This technique, which has been widely used in the earth sciences, permits an examination of the elemental composition of minute volumes (several cubic microns, depending upon density) of intact material. This provides a unique opportunity to examine spatial distribution of elements in fish hard tissue.

The electron microprobe was used in this study to examine (1) various preparations of specimens and operating conditions to determine what procedures are necessary to produce an accurate electron microprobe analysis of calcified tissue of fishes, (2) qualitative and quantitative composition of the elements in pike cleithra, and (3) composition in relation to zonation of calcified tissue from pike and other species.

The microprobe focuses a fine beam of electrons as small as 1 µm in diameter on a highly polished specimen surface which has been covered with a conductive coating. This electron bombardment causes characteristic X-rays to be emitted by the atoms affected by electron excitation. Under conditions of high vacuum, radiation is emitted when an electron pulse falls from an outer shell to fill a vacancy in the inner shell. The energy emitted is equal to the difference in energy of the levels between which the transition takes place. Wavelength and intensity of the emitted X-rays are analyzed by crystal spectrometry, and can be converted to elemental qualitative and quantitative analyses.

This analytical technique provides a sensitivity, depending upon operating conditions and element analyzed, as low as 10 ppm. However, the lower limits are usually in the range of several hundred ppm, with a level of accuracy of approximately ±1% of the quantity present.

Multichannel Electron Microprobe X-ray Analyzers (Applied Research Laboratories, Glendale, California) were used for the analyses.

The electron beam was focused to a spot between 1 to 5 µm in diameter, usually 2 µm. An acceleration voltage of 15 kV was used for all elements except carbon, when 10 kV was used.

Beam current and resulting specimen current and exposure time were examined to determine their effect on count rate and its subsequent conversion to elemental composition.

A time sequence examination of count rates and resultant calcium concentrations with increased beam exposure produced somewhat similar trends in both the opaque and translucent zones. Usually during the first 60 seconds count rates first increased, and then decreased slightly. With continued exposure, count rates showed an increase, followed by a decrease after prolonged exposure. This change was most prominent in the opaque zones.

From this series of tests, it was obvious that, regardless of the effect of current or duration of exposure, calcium concentration was greater in the translucent than in the opaque zone. At higher currents the concentration differed less between zones not only at the end, but also at the beginning, of the analysis. With increasing current, count rate and calcium content increased rapidly at first, but remained constant at specimen currents above 0.20 µA.

To determine which conditions represented an accurate analysis, a cube of adjacent bone which contained these two zones was excised and analyzed for calcium by atomic absorption spectrophotometry. These tests confirmed that the most desirable operating conditions used a specimen current of 0.01 μA, and X-ray intensity measured for the first 10-second counting period immediately after the electron beam impinged on a newly analyzed point gave an accurate microprobe analysis of calcified tissue from fishes.

The effect of surface contamination, mineral concentration, and volatilization of the organic matrix on X-ray production and emission and accurate microprobe analysis of fish calcified tissue are considered.

Wavelength profiles were conducted on a point by scanning a spectrometer crystal through its range. Four types of crystals were used which provided a complete range of wavelengths--lithium fluoride (LiF), ammonium dihydrogen phosphate (ADP), rubidium acid phthalate (RAP), and lead/barium stearate (PB/BA SD).

Elements present in significant quantities appeared as peaks in the spectrum above the background and were identified by reference to a table of X-ray wavelengths. Specific elemental analyses were conducted by experimentally tuning the spectrometers with mineral standards of known analyses of approximately the same elemental composition as those analyzed in the calcified tissue.

Spectrometer scans, throughout the range of wavelengths available for the crystals used, revealed that spectral lines of nine elements (calcium, phosphorus, sodium, magnesium, zinc, sulphur, potassium, chlorine) were readily identified in this series of profiles. In other wavelength scans, characteristic X-ray peaks representing fluorine and silicon were frequently observed above the background.

Quantitative analyses were conducted on pike cleithra for those elements that produced recognizable emission lines in the wavelength profiles or that, according to the literature, occurred in large quantitaties in some calcified tissue of fish.

When a 14-element analysis was conducted, calcium and phosphorus accounted for approximately 95% of the inorganic component in pike cleithra. This analysis of elements heavier than oxygen substantiated that in pike cleithra, calcium and phosphorus were major elements (>1.0%) and sodium, magnesium, zinc, sulphur, and fluorine were minor elements (0.1% to 1.0%). Elements such as chlorine, potassium, silicon, iron, strontium, barium, and aluminum were present, but only in trace amounts (0.1%).

Only seven elements--fluorine, sodium, magnesium, phosphorus, sulphur, potassium, and calcium--were present in concentrations that were clearly within the limits of detection for microprobe analysis. Frequently zinc, silicon, and chlorine occurred in quantities that were within the statistical limits of detection. Semi-quantitative analyses with a lead/barium stearate crystal indicated that carbon, nitrogen, and oxygen were also major elements in pike cleithra.

Initial tests of operating conditions revealed that calcium concentration was greater in translucent than in opaque zones. The most abundant inorganic elements, calcium and phosphorus, varied in concentration from zone to zone. With the operating conditions described, no other elements, with the possible exception of sulphur, showed obvious differences with zonation. The translucent zone usually contained more calcium and phosphorus than did adjacent opaque zones. Calcium and phosphorus were very significantly greater in the translucent than in the opaque zone in cleithral slices from pike from Wickett Lake.

Calcium and phosphorus contents of both the opaque and translucent zones gradually increased with increasing age and decreasing growth rate.

Electron microprobe analyses verified that there were distinct elemental differences between zones and regions of the cleithrum. Differences between zones were usually much less obvious during the first two years of growth than in subsequent years. The translucent zone contained a higher, usually more uniform, calcium concentration than did the opaque zone on either side. The lowest concentrations were often found at the beginning of the opaque zone, and gradually increased across the zone. However, there was considerably more variation than in the translucent zone. The abrupt decrease in calcium concentration at the beginning of the opaque zone was most extreme in the slower growing zones of older cleithra. Under these conditions, an absolute difference of 5 to 6% calcium was occasionally measured between the zones.

Preliminary analyses in relation to zonation indicated that on a weight basis the opaque zone contained slightly more carbon than did the translucent zone.

Otoliths from other species, which contained recognizable optical zones, showed differential mineralization in relation to zonation. Calcium content on a weight basis was slightly greater in the translucent than in the opaque zone. The difference between zones in otoliths were usually not as apparent as those in pike cleithra, although some otoliths from Atlantic cod, Gadus morhua, showed a very striking difference from zone to zone. As in pike cleithra, calcium and mineral contents in otoliths increased with increasing age and decreasing growth rate.

Forensic Applications of SEM/EDX

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Scanning Electron Microscopy - Energy Dispersive X-ray analysis has been used at the Centre of Forensic Sciences since late 1975. In that time approximately 150 cases have involved some aspect of SEM or X-ray analysis. The types of examination have ranged from the identification of arsenic on the copper wire used in the Reisch test to the examination of fracture surfaces in failure analysis.

For the purposes of this paper some cases will be described which illustrate forensic applications of SEM-EDX analysis. Two of the cases are examples of physical matching of broken surfaces and another is SEM-EDX analysis of a small amount of an unusual alloy. Discussion will also be presented on the collection of gunshot residues and subsequent identification of these residues by SEM-EDX.

DETERMINATION OF AIRBORNE AND WATERBORNE ASBESTOS FIBRES BY ANALYTICAL ELECTRON MICROSCOPY

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Detection and identification of asbestos fibres in the environment is a difficult problem. A typical air or water sample contains a variety of minerals and organic debris, particles of which can easily be mistaken for asbestos fibres. Many minerals have good cleavage yielding elongated fragments. These fragments are assigned as fibres when samples are examined by techniques which do not incorporate any identification procedures. In water samples, diatomaceous fragments are common and these also will be included in results from non-specific measurements. The analytical electron microscope (AEM) is the most suitable instrument for measurements on environmental samples since it allows observation of morphology, selected area electron diffraction (SAED) and energy dispersive X-ray analysis (EDXA) for identification of mineral particles even as small as a few nanometres in dimension. Where an absolutely rigorous demonstration must be made of the precise mineral species, all of these techniques must be used, and analyses of electron diffraction data from several crystal orientations are required.

Before analysis is undertaken, the term asbestos must be defined. Asbestos refers to a number of minerals which have the property that they can be separated into very fine, long, silky fibres. The three main kinds of asbestos which have found commercial use are chrysotile, amosite and crocidolite. Of these, chrysotile is by far the most abundant and also the most widely used. Amosite is not in fact a mineral name but is an acronym for Asbestos Mines of South Africa. Amosite usually consists of the amphibole mineral grunerite, but cummingtonite and tremolite are often present in variable proportions. Crocidolite is the mineral sometimes referred to as "blue asbestos". The other, less important, varieties of asbestos are anthophyllite, tremolite and actinolite. Chrysotile is the only asbestos belonging to the serpentine group of minerals; all of the others belong to the amphibole group. Asbestos minerals exhibit variable composition within the same species, and such variations may even occur from one fibre to the next in material from the same deposit.

The asbestoses each have non-fibrous polymorphs of similar composition. The asbestos variety is composed of particles which are extremely thin, have very high length to width ratios and are also very flexible. Particles of the non-asbestos variety usually cleave into needle-shaped fragments frequently of lower aspect ratio and not displaying the property of flexibility. The difference, therefore, between the asbestos and non-asbestos varieties of the same mineral is concerned with a mineralogical property known as crystal habit. Although in a large hand-sized specimen it is relatively simple to discriminate between the crystal habits of the fibrous and non-fibrous versions of the same mineral, this is not true when a single small particle of the mineral is examined in a microscope. It would be attractive to confine the use of the term asbestos to the minerals which are commercially exploited, however this could be misleading. Many mining operations, which do not produce primarily asbestos, have minerals in the ore body which are indistinguishable from asbestos and which are of unknown health hazard. For example, the minerals tremolite and actinolite occur in some deposits of vermiculite, and anthophyllite has been found in some talcs. It is a controversial matter whether such particles of

amphibole should be considered as asbestos. While amphibole is one of the more common minerals in the earth's crust, amphibole crystallized in the asbestiform habit is comparatively rare.

It may be required to measure environmental levels of asbestos in the vicinity of known or suspected sources of asbestos. It may also be necessary to discriminate between fibres discharged from the source, and other particles of an elongated or fibrous nature which may also be present. In many areas remote from sources of asbestos, low levels of asbestos are detected. Unlike samples in asbestos workplaces, the majority of asbestos fibres found in the remote environmental samples are frequently very small (sub-micron) fibres which form a very low proportion of the total fibrous material present, and which are usually below the detection capabilities of the optical microscope. While the scanning electron microscope offers a considerable improvement in resolution, and energy dispersive X-ray analysis can be used to determine the chemical composition of many of the fibres, this instrument is inadequate for discrimination of asbestos fibres from many other particles present in environmental samples.

The analytical electron microscope has a resolution of about 0.2 nm, which is more than adequate for resolving the smallest fibres of asbestos, which are about 40 nm in diameter. The image obtained also permits observations of some internal structure of each fibre. Chrysotile asbestos fibres have a characteristic tubular appearance. Figure 1 is a TEM micrograph showing this chrysotile morphology. The tubular appearance is a result of the scrolled sheet structure of the individual fibre. However, a tubular appearance is not unique to chrysotile; halloysite and palygorskite can exhibit a similar appearance. It has also been shown recently that vermiculite plates can roll into a scroll structure and also give rise to a fibre-like particle which can be confused with chrysotile. The amphibole fibres do not exhibit such a characteristic morphology. Every fibre without tubular morphology and which is not obviously of biological origin, with an aspect ratio of 3 to 1 or greater and having parallel or stepped sides must be considered as a suspected amphibole fibre. SAED and EDXA techniques are required to identify the amphiboles and to confirm the identification of chrysotile asbestos.

Using selected area electron diffraction, a pattern characteristic of the crystalline structure of a fibre is produced. On a modern instrument, facilities are available for tilting and rotating the sample so that the crystalline fibre may be examined along its principal crystallographic directions (zone axes). A typical zone axis pattern obtained for the amphibole crocidolite is shown in Figure 2. Switching between the image and the electron diffraction mode is accomplished simply by adjustment of lens currents and apertures. In investigations where identification of a specific mineral is required, computer analysis of electron diffraction patterns obtained along several principal directions of the crystal is necessary. However, since the crystal structures of tremolite, actinolite, crocidolite and amosite are practically identical, it is not possible using electron diffraction alone to discriminate between these minerals.

Discrimination between the amphibole asbestoses can be reliably achieved only by measurement of the chemical compositions of the fibres. Energy dispersive X-ray equipment incorporated in the TEM allows this composition data to be obtained for each fibre. The EDXA spectrum from a crocidolite fibre is shown in Figure 3. In order to discriminate this material from other species the sodium peak must be present. Figure 4 shows a similar EDXA spectrum from an amosite fibre. However, the chemical information by itself is totally inadequate to permit classification of a fibre as amphibole. Indeed, the chemical information alone even for the simple case of chrysotile asbestos is inadequate for classification, in that the particle may be a fragment of lizardite or antigorite which have similar compositions.

It must be recognized that economic considerations usually preclude unequivocal identification of every fibre reported. The decision as to what

proportion of the fibres must be completely identified is frequently based on how much is known about the source of the sample. Once the presence of asbestos is confirmed, fibres can often be classified on the basis of crystallographic or chemical similarity with identified fibres. Chrysotile is an exception, in that the morphological characteristics in the TEM with some confirmation by SAED and EDXA may be considered adequate for identification by experienced operators.

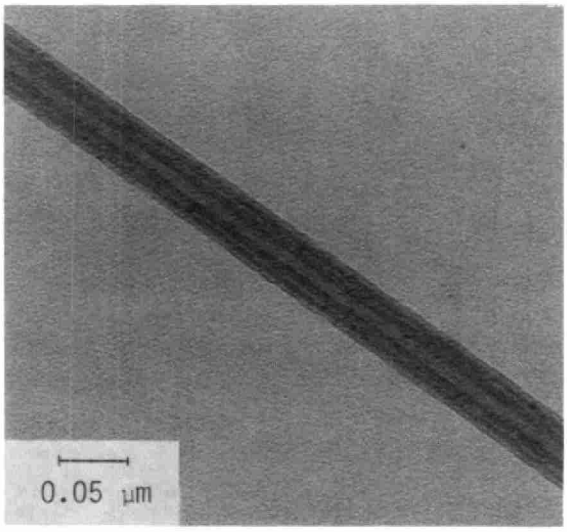

Figure 1. TEM micrograph of chrysotile fibre showing internal morphology.

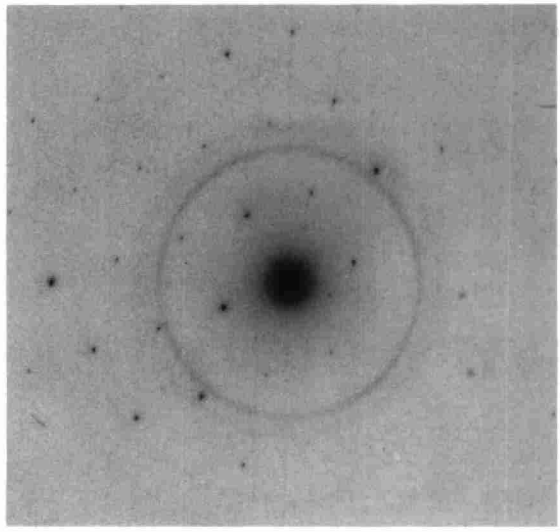

Figure 2. Zone axis SAED pattern of crocidolite fibre.

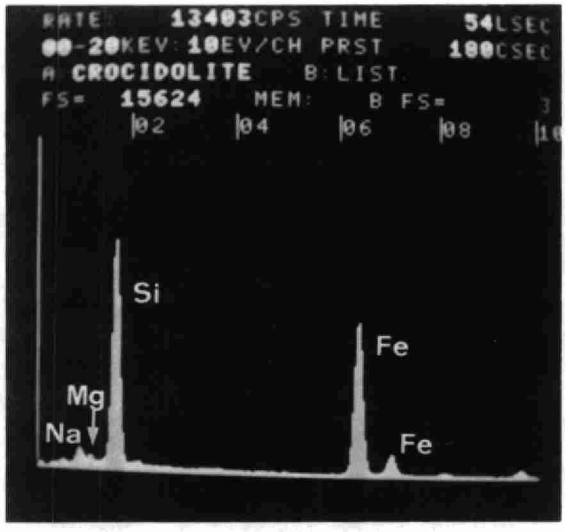

Figure 3. EDXA spectrum of fibre of crocidolite.

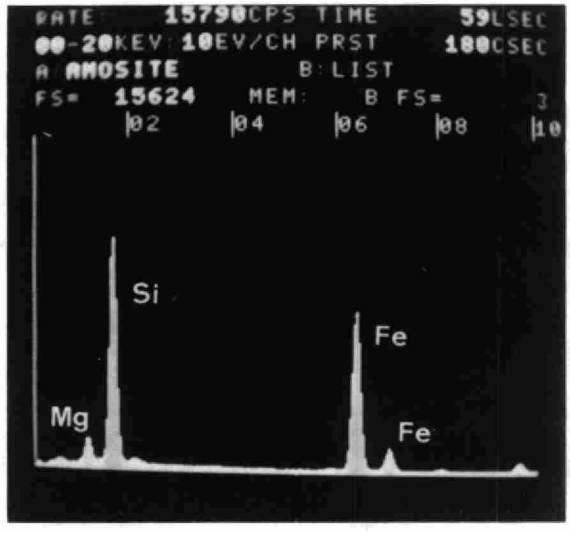

Figure 4. EDXA spectrum of fibre of amosite.

ELECTRON MICROSCOPY IN GEOLOGY AND GEOCHEMISTRY

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X-Ray diffraction analyses is the traditional method of mineralogical analyses. However, this method cannot be used for particles less than 1 micrometer; and it gives no information regarding individual mineral composition or morphology. Electron Microscopy (E.M.) can reveal the morphology, crystallography, and the composition of fine particles.

Minerals characteristically vary in composition between defined limits. The same mineral may have different morphologies and hence different surface properties. Furthermore recent work on alumino-silicate minerals reveals new structures of more than "2-chains". These new structures were revealed by E.M. techniques.

Fine-grained minerals (iron/manganese oxides, clay minerals) result from rock weathering and they are especially suited to E.M. analyses. Often morphology, simple diffractometry and elemental analyses are sufficient to characterize these minerals. E.M. can define the structures of fine grain materials that are amorphous to X-Ray diffraction. A recent study has been carried out on forms of many examples prepared by different chemical recipes for adsorption studies. Three types of morphology were clearly defined by E.M., whereas all these samples gave no X-Ray diffraction patterns.

Minerals often show zones of different composition due to the different processes acting over time. This zoning of greater than 0.1 um can often be analyzed using X-Ray energy dispersive techniques. Similarly reaction rinds of minerals may be analyzed. Normally, a limiting dimension of 0.2 um is required for a quantitative or semi-quantitative estimate.

Analytical methods can be used to separate different minerals of similar morphology or to separate different compositions of the same mineralogy. For example, the fibrous morphology of Mn oxide can be readily differentiated from chrysotile asbestos that has similar morphology. And different compositions of the amphibole mineral series tremolite-actinolite, are readily determined on individual grains.

Trace metals and organics are often found adsorbed on the surface of fine particles or included in the mineral structure. Comparison of trace metal compositions and the specific mineral can be used to define the mode of occurrence. For example, copper associated with kaolinite suggests an adsorption process.

ANALYTICAL ELECTRON MICROSCOPY

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When an electron beam encounters a thin specimen, a large number of different processes can occur which reflect the chemical composition, structure, and thickness of the specimen. The processes include X-ray emission, Auger electron emission, electron diffraction and electron scattering and energy loss processes. Imaging at high resolution has used only the electron scattering and diffraction processes in a qualitative way. Quantitative interpretation of these various effects has led to the concept of analytical electron microscopy in which the quantitative structural and chemical information about the specimen is obtained while the high spatial resolution capability of the transmission electron microscope is maintained. In this paper, only the aspects of qualitative and quantitative analysis and applications of X-ray spectrometry in analytical electron microscopy are discussed.

When an electron beam of high energy strikes a solid specimen, atoms of the specimen will undergo ionization in their inner shells. When returning to the ground state, X-rays characteristic of the kinds of atoms present in the specimen are emitted. The intensity of such emission is low however and it is the development of the solid state (lithium drifted silicon, Si(Li)) detector with good energy resolution which has enabled the practical measurement of X-ray spectra from a thin specimen in a transmission electron microscope. The problem of X-ray microanalysis is that of obtaining a clean spectrum from the area of the specimen bombarded by the electron beam which can be as small as a few tens of nm in diameter and of interpreting the spectrum in terms of the composition of the area bombarded.

The identification of the elements is relatively straightforward. X-ray spectra are simple, with few ambiguities. However, some background contributions and artifacts must be considered. In addition to the characteristic X-ray lines, in the interaction of electrons with atoms, bremstrahlung or continuum X-rays are also generated. These X-rays of all wavelengths are a background on which the characteristic lines are found. Thus, they limit the sensitivity of the technique more than any other factor. At the same time, however, the intensity of the continuum is also used to establish the specimen thickness, a necessary parameter in the quantitative determination of the composition. A more serious problem in qualitative analysis are spurious X-rays generated in parts of the specimen, support grid or electron optical column by electrons scattered from apertures or by X-rays generated when the electrons are stopped by an aperture in the column. These problems can be minimized by careful design of the electron optical column and the X-ray spectrometer and are becoming less significant as newer instruments are built.

For quantitative analysis, provided specimens are thin enough, the X-ray intensity of a characteristic line is just related to the mass of the atoms in the specimen times an ionization probability and X-ray yield. In fact, only relative values for these factors are important. Frequently however, specimens are thick enough that the absorption of X-rays within the specimen must be considered. Criteria for knowing when to apply corrections have been established as well as a number of approaches to the correction itself. Crucial to such corrections is the determination of the mass thickness of the specimen. Bremstrahlung intensity is one means to obtain this thickness which has been particularly useful in biological specimens.

X-ray microanalysis in a transmission electron microscope has been particularly effective in determining the concentration gradients very close to grain boundaries and precipitates and in establishing the composition of small particles. The application of the method is expanding rapidly as the number of STEM and conventional TEM with energy dispersive X-ray spectrometers increases.

AUTOMATION IN ELECTRON MICROSCOPY; APPLICATIONS OF IMAGE ANALYSIS SYSTEMS TO ENVIRONMENTAL PROBLEMS

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The electron microprobe, by virtue of its combined imaging and X-ray analysis capabilities is an extremely versatile instrument for the types of analyses associated with environmental problems. In its most common configuration, with manually operated specimen and beam controls, however, the microprobe suffers from the drawback of requiring considerable time and manual manipulation in order to acquire more than the most rudimentary data on particle size or composition.

Many analytical electron microscopy installations are therefore incorporating image analysis systems into their instruments. Such an automation package is capable of examining the entire field of view of the SEM image. It defines as features those areas whose contrast level exceeds a certain specified threshold as compared with the background. This definition clearly allows the system to consider features such as inclusions in mineral specimens, structural entities within cells, and particles on filters. Two examples of the latter application are shown in Figures 1 and 2 which display, at 1000X and 3000X magnification respectively, air particulate material collected on a Nuclepore membrane filter with pores 0.8μm in diameter.

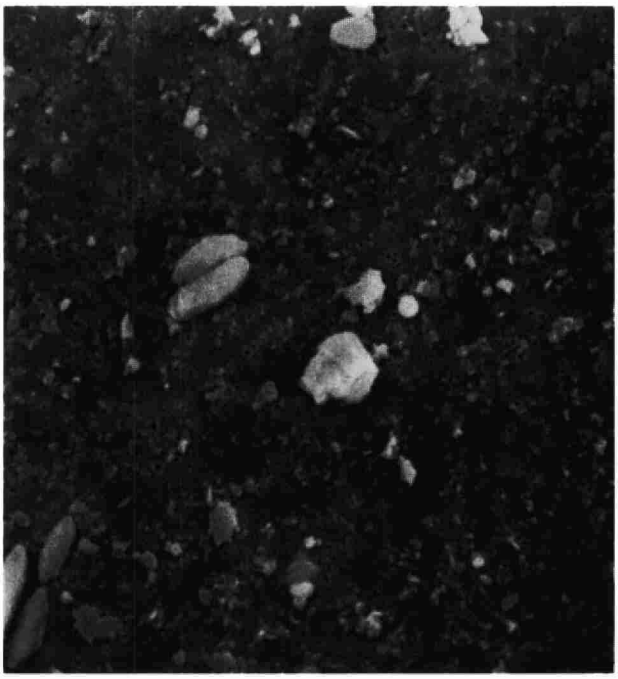

Figure 1. Air Particulate Material
1000X Magnification

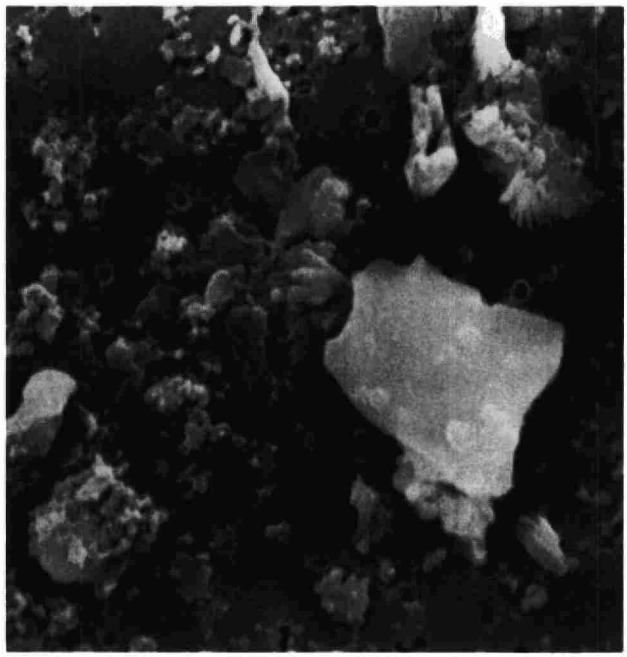

Figure 2. Air Particulate Material
3000X Magnification

The system can be programmed to determine the length, width, perimeter and area of each feature and thereby to determine the particle size distribution. The elemental composition of each particle in turn is determined by having the computer automatically position the electron beam appropriately, acquire the X-ray spectrum and perform the necessary data manipulations. By comparing the observed composition with the known compositions for various types of particles, a summary of results for an air particulate analysis could be presented as shown in Table 1. Having thus analyzed one field of view, the computer can be programmed to move the specimen to the next position and repeat the determination of particle size and composition.

TABLE 1

SIZE DISTRIBUTION (μm)

	PERCENTAGE OF TOTAL PARTICLES				TOTAL PARTICLES
TYPE	0.2-2.5	2.5-5.0	5-15	>15	
FLYASH	94	5	1	0	85
QUARTZ	79	15	5	0	83
Ca-RICH	72	16	12	0	68
Fe-RICH	95	4	1	0	52
Ca-S	99	1	0	0	120
CLAY	85	11	4	0	182
Ca-S	43	40	16	1	70
CARBON	96	4	1	0	75

Such a system is most ideally suited to the examination of flat specimens. One can see immediate applications to studies of bacterial populations, atmospheric and water-borne particulate materials, respirable dusts, fly ash and sediments.

For the case of asbestos analysis, a problem arises which needs special consideration. Although an automatic image analysis system can readily distinguish particles with a 3:1 aspect ratio (the current definition of a fibre), further software refinements have been necessary to deal with the problems of fibre overlap (Figure 3). Systems now exist which can handle that situation remarkably well. Figure 4 shows the output of an automatic interpretation of the image presented in Figure 3. Automatic fibre analysis in the SEM is therefore a reality, at least for occupational samples where a significant fraction of the particulate loading consists of asbestos.

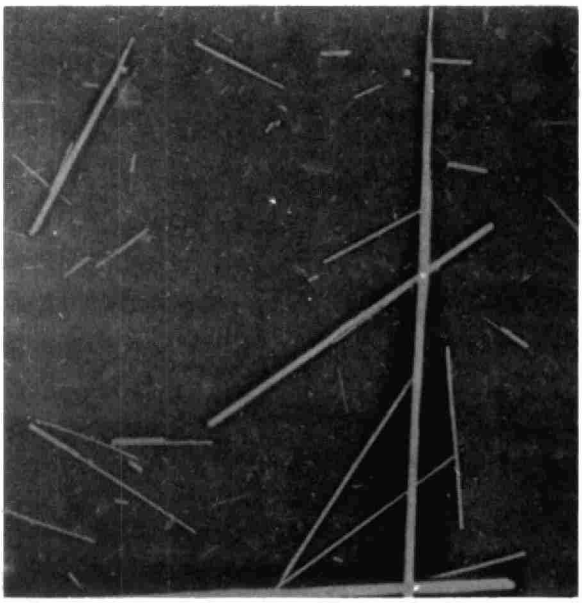

Figure 3. Asbestos Fibres As Seen By SEM

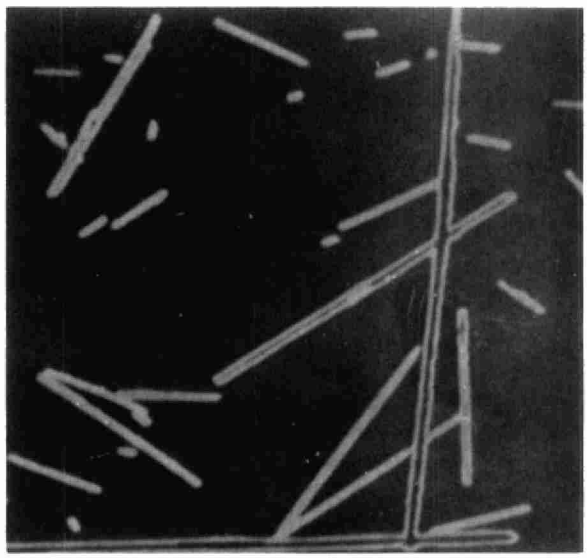

Figure 4. Asbestos Fibres As Recognized By Computer

GLOSSARY

Absorbed Electrons

Those electrons which give up all their energy on collision with the specimen are "absorbed".

Analytical Electron Microscopy (AEM)

Analytical electron microscopy is the term now used to describe the microcharacterization of a material using as much as possible of the information produced by the electron-solid interaction process.

Auger Electrons

Auger electrons are generated by electronic transitions in an atom which occur following excitation of bound electrons.

Backscattered Electrons

When an electron beam strikes a solid specimen, some of the primary electrons are deflected through large angles without energy loss. A portion of these deflected electrons emerge from the surface through which they had initially entered the specimen, and are called backscattered electrons. They escape from depths up to several micrometers. Although most backscattered electrons have energies almost equal to the beam energy, the definition includes all those electrons leaving the surface with energies greater than 50 eV.

Bias

Bias is the grid-to-cathode voltage.

Cathodoluminescence

When certain types of materials are bombarded by high-energy electrons, they emit visible light. This phenomenon is called cathodoluminescence.

Characteristic X-rays

Characteristic X-rays are emitted from an element by the interaction of incident electrons with the inner shell electrons of the atoms constituting the specimen.

Charging

There is a basic requirement in the SEM that the negatively charged beam incident on the sample find some conducting path to ground. If this condition is not satisfied in some way on the surface or within the bulk of the specimen, a condition known as "specimen charging" will occur.

Dark Field Imaging

This is a method of image formation which uses only the electrons scattered by the specimen (which are removed by the objective aperture in normal transmission operation). The main beam passing through the

specimen must be deflected or intercepted so that it cannot reach the final screen; the scattered electrons are directed into the imaging system and form the final image.

Depth of Field

The range of object distances over which structures can still appear reasonably sharp is called the depth of field.

Diffraction

Whenever waves encounter an obstacle or irregularity, new waves are generated which spread out in all directions. This process, which results in a lack of absolute sharpness in shadows, is called diffraction.

Elastically Scattered Electrons

Those electrons which when interacting with a specimen give up a negligible amount of their energy but change their direction are "elastically scattered".

Electromagnetic Lenses

The lenses in the EM are used to demagnify the image of the electron source formed at crossover in the electron gun and to focus the primary beam to a small spot size (usually 10 nm) on the specimen. The condenser lenses determine the beam current that impinges on the specimen while the objective lens determines the final spot size of the electron beam.

The lenses are electromagnetic - an electromagnet consisting of a steel housing and a core of wire windings. It is rotationally symmetric and wound so that electrons are focused toward the central axis of the column. The intensity of the magnetic field produced by the lens is such that electrons travelling down the exact center of the lens are unaffected while electrons displaced from the center are focused toward the central axis.

Electron Energy Loss Spectroscopy (EELS)

Electron energy loss spectroscopy, which is the study of the energy distribution of electrons which have interacted with the specimen as they passed through it, can give quantitative information about the chemical and physical nature of the sample.

Electron Gun

The most commonly used electron gun is composed of three elements: (1) a tungsten wire (the filament or cathode) bent in the form of a V which is surrounded by (2) a metal housing (the cathode shield) which contains an aperture located directly below the filament tip and (3) the anode plate.

The filament is energized by a heating current of a few hundred microamps. When the filament is hot enough, the metal atoms give off a cloud of electrons which tends to be more concentrated at the tip of the filament. The cathode shield is maintained several hundred volts negative with respect to the filament. This negativity ("bias voltage") repels the electrons leaving the filament away from the inner surface of the cathode shield.

Three types of sources are available: (1) the tungsten hairpin filament, (2) lanthanum hexaboride (LaB_6), and (3) field emission.

Electron Microprobe

The electron microprobe and scanning electron microscope are two powerful instruments which permit the characterization of heterogeneous materials and surfaces on a local scale. In both instruments, the area to be examined is irradiated with a finely focused electron beam, which may be static, or swept in a raster across the surface of the specimen. In the electron microprobe, the primary signal of interest is the characteristic X-rays which are emitted as a result of the electron bombardment. The analysis of the characteristic X-radiation yields compositional information of both a qualitative and quantitative nature.

Electrons

Electrons are negatively charged particles which, from a simplified point of view, can be considered to be located in planetary orbits around atomic nuclei.

They are prevented from escaping from these orbits by the strong attractive force created by the positively charged nucleus.

Energy Dispersive X-ray Analysis (EDX)

X-rays, being electromagnetic radiation, have measurable wavelengths and energies. In an energy dispersive analysis sytem the characteristic X-rays emitted by the specimen deposit their energy within a solid state detector. A pulse height/multichannel analyzer can display the number of X-ray impulses of each energy thereby indicating which elements are present in the specimen (cf. Wavelength Dispersive Spectroscopy).

Fluorescence

Fluorescence is the property of emitting electromagnetic radiation under the influence of electron beam bombardment. Certain materials emit visible light under these conditions.

This phenomenon is useful in electron microscopy for making the electron image visible. A screen coated with a material capable of fluorescence in the visible range emits visible light when bombarded with electrons.

Inelastically Scattered Electrons

Those electrons which interact with the specimen by giving up part of their energy with negligible change in direction are "inelastically scattered".

Resolving Power

The smallest distance by which two points on a specimen can be separated and still be distinguished as two points.

The maximum theoretical resolving power of a microscope is about half the wavelength of the illuminating beam.

Secondary Electrons

By convention, electrons that escape from the specimen surface with less than 50 eV are designated secondary electrons. The number of secondary electrons emitted from the specimen surface depends upon the energy and direction of the primary electrons and the density of the specimen.

Signal-to-Noise Ratio (SNR)

The signal-to-noise ratio is defined as the ratio between the rms signal and the rms fluctuation attributable to noise. The effective SNR is that part of the total signal that is employed to modulate the display CRT (cathode ray tube) from black to white.

Wavelength Dispersive Spectroscopy (WDS)

Wavelength dispersive spectrometers measure the wavelengths of X-rays produced when an electron beam hits a sample. X-rays are reflected by a crystal of known lattice spacing. Since only one particular wavelength is strongly reflected at a certain angle, it is possible to calculate the wavelength of the X-rays. With this system only one wavelength can be measured at one time (cf. Energy Dispersive X-ray Analysis).

Working Distance

Working distance is the distance from the bottom pole-piece of the objective lens to the specimen surface. This distance ranges from 5 to 25 mm in most instruments.

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